EFFECTS OF ACCLIMATION AND EXPERIENCE TO CHANGING HEAT AND COLD SHOCK TEMPERATURE ON LETHAL TEMPERATURE AND THERMAL TOLERANCE OF *GAMBUSIA AFFINIS* (BAIRD & GIRARD) (POECILIIDAE)

by

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ABSTRACT. - The effect of body size and acclimation temperatures on the lethal temperatures as well as the effect of exposure to a range of experimental temperatures on the thermal tolerance of *Gambusia affinis* were studied. The tolerance to both upper and lower experimental temperatures were increased with increasing the body size (standard length, SL) from 1.5 to 3.5 cm. Fish acclimated to 10, 20 and 30°C showed normal heat acclimation since their LT-50 were 32.0, 36.5 and 38.5°C, respectively, The amount of increased heat tolerance for each 5°C increase in the acclimation temperature was 2°C between 10 and 20°C and 1°C between 20 and 30°C. Fish acclimated to 10,20 and 30°C showed normal cold acclimation and their lower LT-50 were 1.0, 4.0 and 7.0°C respectively. The amount of reduced cold tolerance temperature during cold acclimation was 1.5°C for each 5°C decrease in the acclimation temperature. The effect of exposure to changing lethal temperatures did not followed a particular pattern. In about 47% of the experiments, the fish gained heat tolerance where as in the same percentage (47%) of the experiment, the fish lost heat tolerance. In the remaining 6% of the experiments the fish were not influenced by changing lethal temperatures.

RÉSUMÉ. - L'influence de la taille et de l'acclimatation à la température sur les températures léthales ainsi que l'influence de l'exposition au changement de température sur la résistance thermique de *Gambusia affinis* ont été étudiées. La résistance aux températures léthales, hautes et basses, est augmentée lorsque la taille du corps augmente (LS de 1,5 à 3,5cm). Les poissons acclimatés à 10, 20 et 30°C présentent une acclimatation raisonnable à la chaleur puisque leurs températures léthales (LT) sont de 32,0, 36,5 et 38,5°C respectivement. Le gain de résistance à la chaleur pour chaque augmentation de la température d'acclimatation est de 2°C entre 10 et 20°C et de 1°C entre 20 et 30°C. Les poissons acclimatés à 10, 20 et 30°C présentent une acclimatation raisonnable au froid et leurs LT minimales sont de 1,0, 4,0 et 7,0°C respectivement. Le gain de résistance au froid pendant l'acclimatation au froid est de 1,5°C pour chaque diminution de 5°C. L'effet d'une exposition à des changements des températures léthales ne suit pas une loi particulière. Dans environ 47% des expérimentations, les poissons ont augmenté leur résistance à la chaleur, mais dans ce même pourcentage les poissons ont perdu leur résistance à la chaleur. Dans les 6% restant, les poissons n'ont pas été influencés par le changement des températures léthales.

Key-words. - Poeciliidae, *Gambusia affinis*, Acclimation, Lethal temperature, Heat death, Cold death, Changing lethal temperature, Survival.

The mosquito fish *Gambusia affinis* is a tropical fish which has been introduced recently into inland waters of Iraq to be used for the biological control of mosquito larvae. *Gambusia affinis* is a eurythermal species which can tolerate a wide range of environmental temperatures (Winkler, 1975). Most of the studies on resistance

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acclimation in fish are concerned with the effect of constant temperatures on their thermal resistance (Precht *et al.*, 1973; Lee, 1980; Al-Habbib, 1981; Threader and Houston, 1983; Bulger and Tremaine, 1985; Hokanson and Koents, 1986; Shahir, 1989). There have been only few studies to investigate the effect of exposure to changing lethal temperatures on the thermal resistance of fish. Research on the young speckled trout *Salvelinus fontinalis* (Fry *et al.*, 1946), *Chalcalburnus chalcoides* (Al-Habbib, 1981) and *Cyprinion macrostomus* (Al-Habbib and Al-Habbib, 1979; Al-Habbib and Shahir, 1991) are examples. In Iraq *Gambusia affinis* inhabits inland water that are subject to a wide range of environmental temperature (from 10°C or less during winter to about 30°C during summer). To date, no attempt has been made to investigate any aspect of the environmental physiology in this species since its introduction. Accordingly the first part of this project was undertaken to investigate the effect of acclimation and changing lethal temperatures on upper and lower lethal temperatures and thermal tolerance in *Gambusia affinis*.

MATERIALS AND METHODS

Individuals of *Gambusia affinis* used during this investigation were collected from Al-Khawsar river (the section passing through Mosul city) during the period between June, 1986 and July, 1987. After collection, the fish were transferred to the laboratory and placed in glass aquaria (50x30x30 cm) containing dechlorinated water and aerated with Hi-Flo aerators. Each aquarium was stocked with 40 fishes. The temperatures of the aquaria were set at 10, 20 and 30°C using heaters connected to contact thermometers and electronic relays. The fish were kept under controlled photoperiod of 12 h light followed by 12 h of darkness. Due to the faster rate of acclimation to high temperatures, they were acclimated to 10, 20 and 30°C for 30, 21 and 15 days, respectively. The fish were fed with commercial fish food. The water of the aquaria was changed once every 2-4 days (depending upon the acclimation temperature).

The upper and lower lethal temperatures of the fish were determined by exposing groups of ten fish for 60 min to a series of experimental temperatures. The water was oxygenated continuously by bubbling a stream of pure oxygen. Five replicate groups were exposed to each temperature. After the recovery period (2 h at 20°C), the percentage of mortality was plotted against the lethal temperature and the upper and lower LT-50 (the lethal temperatures that cause 50% mortality in a giver population) were calculated (Fig. 1, 3).

The effect of body size on the thermal tolerance was investigated by exposing groups of fish acclimated to 25°C at various temperatures between 33 to 40°C and 2 to 9°C. The average standard lengths of small, medium and large size fishes were 15, 25 and 35 mm, respectively. The effect of acclimation temperature on the upper and lower lethal temperatures was studied by exposing groups of fish acclimated to 10, 20 and 30°C to various temperatures between 28 to 41°C and 0 to 10°C.

The effect of exposure to changing lethal temperatures was determined by the method described by Al-Habbib and Al-Habbib (1979) in which groups of ten individuals were exposed to a range of lethal temperature combinations for different lengths of time (Table I). The lethal temperature combination as well as the exposure time were selected on the basis of these mean survival duration-temperature experiments. The fish were kept at the last lethal temperature until they had all died. Finally, the mean observed duration was calculated and the accumulated lethal doses, which were the lethal doses required to bring about mortality, were estimated by adding together the lethal fractions which were determined as follows:

$$\text{Lethal fraction at t°C} = \frac{\text{exposure time (min)}}{\text{mean survival duration at t°C (min)}}$$

Acclimation temperature	Experiment No.	Sequence and duration of temperature change				Observed duration (minutes)			Theoretical duration (min)	Accumulated lethal doses %
						Mean	S.E.M.*	n*		
10°C	1	31° (19 min)	32° (15 min)	33° (5 min)	34° until last death	41.00	3.21	10	35.117	125.19
	2	31° (12 min)	33° (11 min)	34° (6 min)	35° until last death	23.00	2.75	10	32.10	68.84
	3	34° (10 min)	32° (3 min)	33° (8 min)	31° until last death	33.40	9.64	10	30.12	128.31
	4	31° (17 min)	33° (10 min)	32° (2 min)	35° until last death	26.95	3.87	10	34.81	79.391
	5	34° (10 min)			33° until last death	10.20	3.20	10	33.59	27.37
20°C	1	36° (15 min)	37° (30 min)	38° (45 min)	39° until last death	56.02	5.82	10	131.19	105.15
	2	36° (15 min)	38° (19 min)	39° (25 min)	39° until last death	34.25	3.29	10	43.37	36.96
	3	39° (1.5 min)	37° (2 min)	38° (8 min)	36° until last death	17.25	4.52	10	22.34	132.03
	4	36° (12 min)	38° (17 min)	37° (24 min)	40° until last death	42.94	6.41	10	74.33	60.10
	5	39° (65 min)	38° (1 min)	37° (1.5 min)	36° until last death	74.40	3.16	10	131.13	92.92
30°C	1	37° (60 min)	38° (65 min)	39° (13 min)	40° until last death	121.05	11.18	10	144.62	87.62
	2	37° (60 min)	39° (65 min)		40° until last death	198.41	12.01	10	252.99	157.19
	3	41° (12 min)	40° (3 min)	38° (6 min)	39° until last death	194.50	22.42	10	148.02	398.51
	4	37° (90 min)	39° (21 min)		38° until last death	115.70	13.84	10	99.52	97.75
	5	40° (14 min)	37° (45 min)	41° (7.5 min)	39° until last death	63,00	12.61	10	86.05	108.52

Table 1. - Accumulated thermal exposure at a range of lethal temperatures in the mosquito fish, *Gambusia affinis*, acclimated to 10, 20 and 30°C.
* Standard error of mean; ** number of observations.

RESULTS AND DISCUSSION

In *Gambusia affinis*, the upper lethal temperature was size dependent (Fig. 1). The large size fish (SL 3.5 cm) were more tolerant when compared with small size fish (SL 1.5 cm). Statistical analyses of the results using ANOVA test (Schefler, 1969) revealed that the LT-50 increased significantly with the body size (F is significant at 5% level). A similar relation has been reported for a very few other fish species such as *Micropterus salmoides* (Guest, 1985).

The cold tolerance was also increased significantly with increasing body size (F is significant at 5% level) (Fig. 2). Thus as a result of increasing body size of *G. affinis*, both upper and lower thermal tolerance are increased and so the total temperature tolerance range is increased. A similar relation between body size and cold tolerance has been observed in *Oncorhynchus* (Brett, 1952), *Coregonus artedii* (Edsall and Colby, 1970) and *C. chalcoides* (Al-Habbib, 1981).

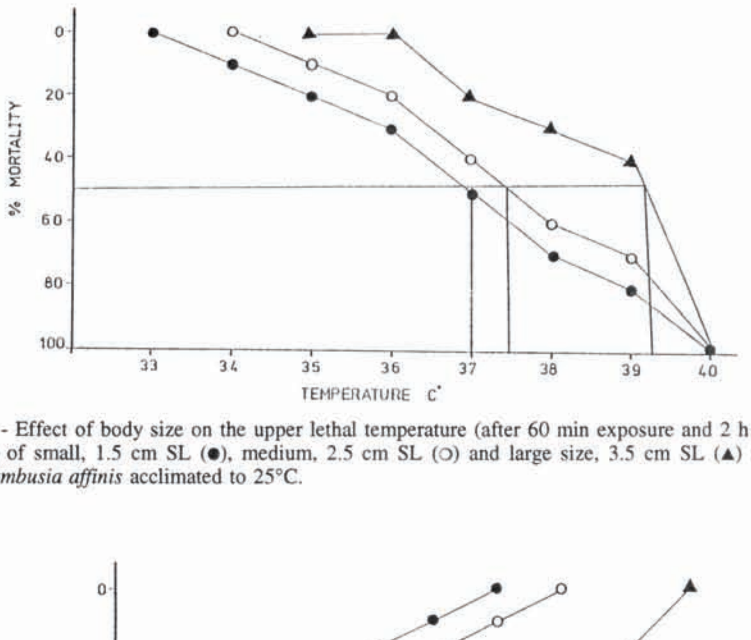

Fig. 1. - Effect of body size on the upper lethal temperature (after 60 min exposure and 2 h recovery period) of small, 1.5 cm SL (●), medium, 2.5 cm SL (○) and large size, 3.5 cm SL (▲) mosquito fish *Gambusia affinis* acclimated to 25°C.

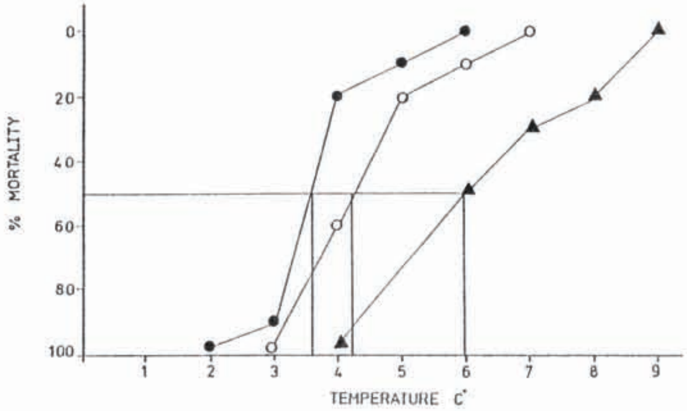

Fig. 2. - The effect body size on the lower lethal temperature (after 60 min exposure and 2 h recovery period) of small (●), medium (○) and large size (▲) *G. affinis* acclimated to 25°C.

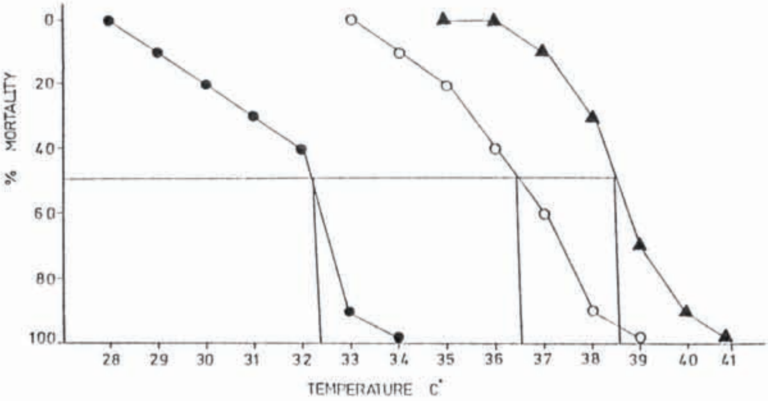

Fig. 3. - The effect on the upper lethal temperature of acclimation temperatures 10°C (●), 20°C (○) and 30°C (▲) on *G. affinis*.

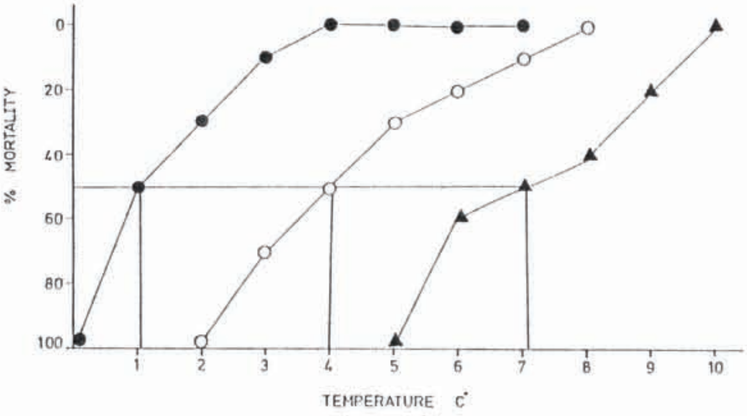

Fig. 4. - The effect on the lower lethal temperature of acclimation temperatures 10°C (●), 20°C (○) and 30°C (▲) on *G. affinis*.

The upper lethal temperature is increased significantly (F is significant at 1% level) as a result of increasing the acclimation temperature from 10 to 30°C (Fig. 3). The rate of heat tolerance gain was 4°C per 10°C between the acclimation temperatures 10 to 20°C and 2°C per 10°C between 20 and 30°C. Increase of heat resistance with acclimation temperature was found in *G. affinis* (Winkler, 1975); *Cyprinion macrostomus* (Al-Habbib and Al-Habbib, 1979); *Chalcalburnus chalcoides* (Al-Habbib, 1981); *Coregonus artedii* (Edsall and Colby, 1970), and in some tropical fish species (Jeffery, 1973), *Cyprinodon* (Otto and Gerking, 1973) and *Micropterus salmoides* (Guest, 1985).

Gambusia affinis also showed normal cold acclimation since their lower LT-50 were decreased significantly (F is significant at 1% level) by lowering the acclimation temperature (Fig. 4). The rate of cold tolerance gain was 3°C per 10°C as a result of decreasing the acclimation temperature from 30 to 10°C. In *Chalcalburnus chalcoides*, the rate of gain was 2.0-3.0°C per 10°C (Al-Habbib, 1981). A similar response to cold acclimation has been shown in other fish species such as *Coregonus artedii* (Edsall and Colby, 1970) and *Micropterus salmoides* (Contant, 1983).

The overall relation between the acclimation temperature and the lethal temperature in *G. affinis* is known as normal heat acclimation - normal cold acclimation (Precht, 1967). Figure 5 shows that temperature tolerance range remained unchanged when the acclimation temperature is increased from 10 to 30°C. Normal heat acclimation - normal cold acclimation could have a great significance in withstanding extreme temperatures resulted from the seasonal variation in the habitat temperature. Furthermore, the rate of improved heat resistant exhibited by *G. affinis* is high when compared with other fish species and this plays a significant role in adapting to thermal changes at both high and low environmental temperatures.

The area of tolerance (expressed as a unit of tolerance being equal to the area bounded by 1°C and expressed as degree centigrade squared, Brett, 1956) was 633.3 units. In *Chalcalburnus chalcoides*, the tolerance value was 640 units (Al-Habbib, 1981). It was 625 units in *Salvelinus fontinalis* (Fry *et al.*, 1946), 450 to 529 units in Salmonid fish (Brett, 1952), 740 to 940 units in some Cyprinid species (Hart, 1974) and 970-1162 units in some Ameuirids (Hart, 1952).

Figure 6 shows the mean survival duration-temperature curve of *G. affinis* acclimated to 10, 20 and 30°C exposed to various experimental temperatures between 31 to 42°C. The mean survival duration of the fish depends upon the exposure as well as the acclimation temperature. The mean survival duration was increased by increasing the acclimation temperature. In addition, the mean survival duration at the lower level of the upper lethal temperature was higher than its duration at the upper level of the upper lethal temperature. Thus the LT-50 (the time of exposure that cause 50% mortality in the population) for 20°C acclimated fish at 36, 37, 38 and 39°C were 118.8, 39.0, 12.5 and 6.0 minutes, respectively.

The effect of exposure to upper changing lethal temperatures on the thermal resistance of fish acclimated to 10, 20 and 30°C are shown in table I. In fish acclimated to 10°C exposure to changing lethal temperature caused an increase in the thermal resistance of the fish in 40% of the experiments since they died after receiving 125 to 128% of the lethal doses. On the other hand, in the remaining 60% of the experiments, the fish lost thermal resistance and died after receiving only 27 to 79% of the lethal doses. The same pattern of response was observed in fish acclimated to 20°C. The response of 30°C acclimated fish to changing lethal temperature was quite different, since the fish in most of the experiments, either gained heat resistance (in 60%) and died after receiving 108 to 398% of the lethal doses or were not influenced by changing lethal temperature (in 20%) and died after receiving 98% of the lethal doses. On the other hand, in only 20% of the experiments, the fish lost heat resistance and died after receiving 87% of the lethal doses.

From these results, it can be concluded that the effect of experience to changing lethal temperatures did not followed a particular pattern. In 47% of the total experiments, the fish gained heat resistance and in the same percentage (47%), the fish lost heat resistance. In the remaining experiments (6%), the fish were not influenced by changing lethal temperature. However, the effect of exposure to changing lethal temperature in fish acclimated to 30°C were more significant since in 60% of the experiments the fish gained heat resistance. This has ecological significance in fish acclimated to warm water during summer months. In fish acclimated to 10 or 20°C, it seems that changing lethal temperature has no effect on *Gambusia affinis*. This may be the result of the large difference between the acclimation and lethal temperatures.

The effect of exposure to changing lethal temperature in fish has been studied to a very limited extent and so there are few results to compare with those produced for *G. affinis*. However, in the speckled trout *Salvelinus fontinalis*, it has been found that changing lethal temperature has no significant effect and fish died after receiving 91 to 100% of the lethal doses (Fry *et al.*, 1946). On the other hand, it has been found that

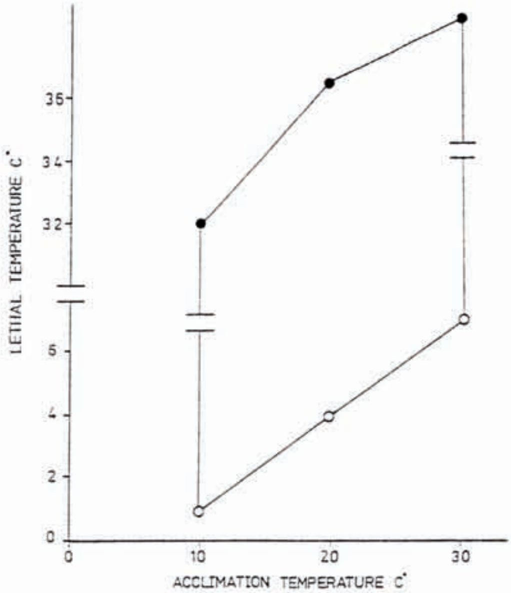

Fig. 5. - A trapezium polygon showing the relationship between the acclimation and lethal temperatures. Solid and open circles represent the upper and lower LT-50 in *G. affinis*.

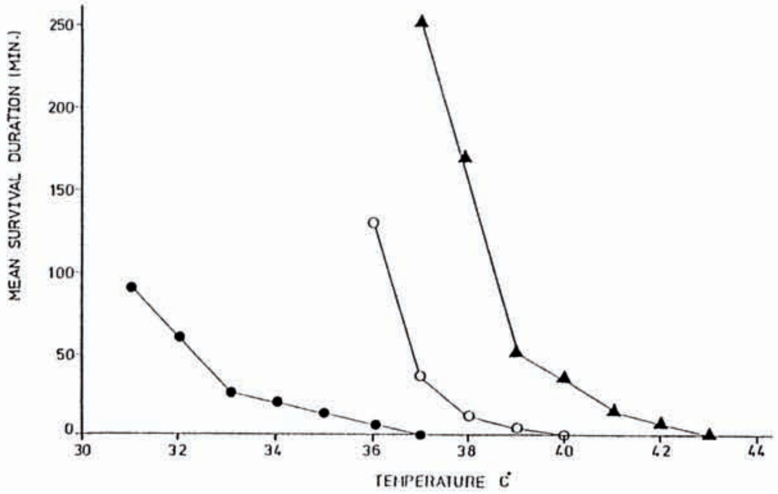

Fig. 6. - Time-temperature curve of *G. affinis* acclimated to 10°C (●), 20°C (○) and 30°C (▲).

changing lethal temperature has significant effects on *Cyprinion macrostomus* (Al-Habbib and Al-Habbib, 1979) and in *Chalcalburnus chalcoides* (Al-Habbib, 1981). In both species, it has been found that the effect depends upon the starting temperature and the sequence of temperature change. In experiments in which the starting temperature ranged from 32 to 34°C and the temperature was varied in an ascending order, their thermal resistance was increased whereas in experiments in which the starting temperatures ranged from 36 to 40°C and the temperature varied in a descending order, the fish lost heat tolerance. However, in *Gambusia affinis* no such relation existed between the starting temperature and the sequence of temperature change and thermal tolerance of this species.

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